https://doi.org/10.33380/2305-2066-2022-11-2-174-179
UDC 615.22

Research article / Оригинальная статья

Study of the role of heparin in regulation of the morphofunctional properties of MSC *in vitro*

Igor K. Norkin[1]✉, Kristina A. Yurova[1], Olga G. Khaziakhmatova[1], Elena S. Melashchenko[1], Vladimir V. Malashchenko[1], Egor O. Shunkin[1], Aleksander N. Baikov[2], Igor A. Khlusov[2], Larisa S. Litvinova[1]

[1] Center of Immunology and Cellular Biotechnology, Immanuel Kant Baltic Federal University, 6, Gaidar str., Kaliningrad, 236041, Russia
[2] Siberian State Medical University, 2, Moskovsky tract, Tomsk, 634050, Russia

✉ **Corresponding author:** Igor K. Norkin. **E-mail:** norkin_igor@mail.ru

ORCID: Igor K. Norkin – https://orcid.org/0000-0002-5212-2435; Kristina A. Yurova – https://orcid.org/0000-0001-6146-3330; Olga G. Khaziakhmatova – https://orcid.org/0000-0002-5525-3529; Elena S. Melashchenko – https://orcid.org/0000-0002-8987-0664); Vladimir V. Malashchenko – https://orcid.org/0000-0003-3437-0926; Egor O. Shunkin – https://orcid.org/0000-0003-0326-0409; Aleksander N. Baikov – https://orcid.org/0000-0002-6321-7595; Igor A. Khlusov – https://orcid.org/0000-0003-3465-8452; Larisa S. Litvinova – https://orcid.org/0000-0001-5231-6910.

Received: 18.03.2022 **Revised:** 25.04.2022 **Published:** 25.05.2022

Abstract

Introduction. Artificial materials used in regenerative medicine induce a balanced inflammatory response after implantation, which is an important step for effective regeneration of damaged bone tissue. The contact of the implant with tissues and biological fluids is accompanied by the deposition of blood proteins on its surface, which contributes to the activation of the complement system and initiates blood clotting, leading to the formation of a fibrin clot. On the surface of the implant, fibrin ensures the adhesion of stem cells and their maturation into fibroblasts that produce collagen and its derivatives. The formed extracellular matrix is the basis for the formation of a tissue structure (callus). To prevent the development of postoperative pathological conditions caused by hypercoagulatory syndrome, therapeutic strategies with anticoagulants such as heparin are used. However, their use limits the formation of a fibrin clot in vivo, which may slow down the migration of mesenchymal stromal cells (MSCs) and the subsequent formation of callus.

Aim. To investigate of the effect of heparin at pharmacological concentrations on stemness and the ability of MSCs from human adipose tissue to undergo osteogenic differentiation under conditions of in vitro cultivation.

Materials and methods. To assess the morphofunctional state of cells cultured in the presence of heparin, 2 experimental groups were formed: 1) MSCs in the presence of heparin at a therapeutic concentration (1.3 IU/ml); 2) MSCs in the presence of heparin at a toxic concentration (13 IU/ml).

Results and discussion. Flow cytometry results showed that the addition of heparin at both concentrations used in the study to MSC culture leads to an increase in the number of cells expressing the surface markers CD73 and CD90, indicating the maintenance of their stem state. On the other hand, a stimulatory effect of heparin at both concentrations used on the transcription of mRNA of osteogenic genes (BMP2, BMP6, ALPL, RUNX2, BGLAP and SMURF1) in MSCs was also observed, which may indicate the osteogenic potential of heparin for the cell culture studied.

Conclusion. The results of the study are useful for regenerative medicine related to the use of MSCs in clinical practice; they may serve as a prerequisite for the development of new therapeutic strategies for orthopedic and traumatologic patients at high risk of postoperative thrombosis after endoprosthetics surgery and osteosynthesis.

Keywords: MSC, heparin, in vitro, expression of osteodifferentiation genes, markers of stemness

Conflict of interest. The authors declare that they have no obvious and potential conflicts of interest related to the publication of this article.

Contribution of the authors. Igor K. Norkin, Kristina A. Yurova and Olga G. Khaziakhmatova adapted, developed and carried out experimental cultivation of MSC. Igor K. Norkin, Elena S. Melashchenko, Vladimir V. Malashchenko and Egor O. Shunkin carried out the research using the instrumental methods presented in the paper and also participated in the creation of a database and statistical processing of the results. Igor K. Norkin, Kristina A. Yurova, Aleksander N. Baikov, Igor A. Khlusov and Larisa S. Litvinova participated in writing the text of the article and its revision. All authors participated in the discussion of the results.

Funding. State assignment in the field of scientific activity [No. FZWM-2020-0010 to Larisa Litvinova].

Acknowledgment. The authors are grateful to the Siberian State Medical University development program Priority 2030.

For citation: Norkin I. K., Yurova K. A., Khaziakhmatova O. G., Melashchenko E. S., Malashchenko V. V., Shunkin E. O., Baikov A. N., Khlusov I. A., Litvinova L. S. Study of the role of heparin in regulation of the morphofunctional properties of MSC *in vitro*. *Drug development & registration*. 2022;11(2):174–179. (In Russ.) https://doi.org/10.33380/2305-2066-2022-11-2-174-179

Изучение роли гепарина в регуляции морфофункциональных свойств МСК *in vitro*

И. К. Норкин[1]✉, К. А. Юрова[1], О. Г. Хазиахматова[1], Е. С. Мелащенко[1], В. В. Малашенко[1], Е. О. Шункин[1], А. Н. Байков[2], И. А. Хлусов[2], Л. С. Литвинова[1]

[1] Центр иммунологии и клеточной биотехнологии Балтийского федерального университета им. И. Канта, 236041, Россия, г. Калининград, ул. Гайдара, 6
2 ФГБОУ ВО «Сибирский государственный медицинский университет» Министерства здравоохранения Российской Федерации (ФГБОУ ВО СибГМУ Минздрава России), 634050, Россия г. Томск, Московский тракт, д. 2

✉ **Контактное лицо:** Норкин Игорь Константинович. **E-mail:** norkin_igor@mail.ru

ORCID: И. К. Норкин – https://orcid.org/0000-0002-5212-2435; К. А. Юрова – https://orcid.org/0000-0001-6146-3330; О. Г. Хазиахматова – https://orcid.org/0000-0002-5525-3529; Е. С. Мелащенко – https://orcid.org/0000-0002-8987-0664); В. В. Малашенко – https://orcid.org/0000-0003-3437-0926; Е. О. Шункин – https://orcid.org/0000-0003-0326-0409; А. Н. Байков – https://orcid.org/0000-0002-6321-7595; И. А. Хлусов – https://orcid.org/0000-0003-3465-8452; Л. С. Литвинова – https://orcid.org/0000-0001-5231-6910.

Статья поступила: 18.03.2022 **Статья принята в печать:** 25.04.2022 **Статья опубликована:** 25.05.2022

Резюме

Введение. Искусственные материалы, применяемые в регенеративной медицине, при имплантации индуцируют развитие сбалансированной воспалительной реакции, что является ключевым этапом для эффективной регенерации поврежденной костной ткани. Контакт имплантата с тканями и биологическими жидкостями сопровождается осаждением белков крови на его поверхности, что способствует активации системы комплемента и инициирует коагуляционный гемостаз, приводящий к образованию фибринового сгустка. На поверхности имплантата фибрин обеспечивает адгезию стволовых клеток, их созревание в фибробласты, продуцирующие коллаген и его производные. Образующийся внеклеточный матрикс лежит в основе формирования тканевой структуры (костной мозоли). Для предотвращения развития постоперационных патологических состояний, вызванных гиперкоагуляционным синдромом, используют терапевтические стратегии с применением антикоагулянтов, таких как гепарин. Однако их использование ограничивает образование сгустка фибрина in vivo, что может замедлять миграцию мультипотентных мезенхимальных стромальных клеток (ММСК) и последующее формирование костной мозоли.

Цель. Изучение влияния гепарина в фармакологических концентрациях на «стволовость» и способность ММСК жировой ткани человека к остеогенной дифференцировке в условиях культивирования *in vitro*.

Материалы и методы. Для оценки морфофункционального состояния клеток, культивируемых в присутствии гепарина, было сформировано 2 экспериментальные группы: 1) ММСК в присутствии гепарина в терапевтической концентрации (1,3 МЕ/мл); 2) ММСК в присутствии гепарина в токсической концентрации (13 МЕ/мл).

Результаты и обсуждение. По результатам проточной цитометрии было выявлено, что добавление гепарина в обеих используемых в исследовании концентрациях в культуру ММСК приводит к увеличению числа клеток, экспрессирующих поверхностные маркеры CD73 и CD90, что свидетельствует о сохранении их стволового состояния. С другой стороны, выявлено стимулирующее действие гепарина также в обеих используемых концентрациях на транскрипцию в ММСК мРНК генов остеодифференцировки (BMP2, BMP6, ALPL, RUNX2, BGLAP и SMURF1), что может указывать на остеогенный потенциал гепарина для исследуемой культуры клеток.

Заключение. Результаты исследования полезны для регенеративной медицины, связанной с использованием ММСК в клинической практике; могут служить предпосылкой для разработки новых терапевтических стратегий для пациентов ортопедотравматологического профиля с высоким риском развития послеоперационных тромбозов после проведения эндопротезирования и остеосинтеза.

Ключевые слова: МСК, гепарин, in vitro, экспрессия генов остеодифференцировки, маркеры стволовости

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Вклад авторов. И. К. Норкин, К. А. Юрова и О. Г. Хазиахматова адаптировали, разработали и провели экспериментальное культивирование МСК. И. К. Норкин, Е. С. Мелащенко, В. В. Малашенко и Е. О. Шунькин провели исследование с использованием представленных в статье инструментальных методов, а также участвовали в создании базы данных и статистической обработке результатов. В написании текста статьи и ее доработке участвовали И. К. Норкин, К. А. Юрова, А. Н. Байков, И. А. Хлусов и Л. С Литвинова. В обсуждении результатов участвовали все авторы.

Финансирование. Государственное задание в области научной деятельности [No. FZWM-2020-0010 Литвиновой Ларисы].

Благодарность. Авторы выражают благодарность программе развития СибГМУ «Приоритет 2030».

Для цитирования: Норкин И. К., Юрова К. А., Хазиахматова О. Г., Мелащенко Е. С., Малашенко В. В. , Шункин Е. О., Байков А. Н., Хлусов И. А., Литвинова Л. С. Изучение роли гепарина в регуляции морфофункциональных свойств МСК *in vitro*. *Разработка и регистрация лекарственных средств*. 2022;11(2):174–179. https://doi.org/10.33380/2305-2066-2022-11-2-174-179

INTRODUCTION

Implantable artificial materials and structures used in regenerative medicine for osteosynthesis elicit an inflammatory response upon contact with body tissue. The development of local inflammation is a necessary process for effective regeneration of damaged bone tissue [1]. Contact of the implanted material with the recipient's tissues enhances this process and is accompanied by the deposition of a layer of proteins on its surface that initiate the complement system, components of innate immunity, resulting in the activation of blood

clotting and the formation of a fibrin clot (hematoma) [2]. The protein scaffold is embedded in a negatively charged matrix of sulfated glycosaminoglycans (GAGs) such as heparan sulfate and others, which interact with platelet-derived growth factors, vascular endothelial growth factor (VEGF) and transforming growth factor-β (TGF-β). This reduces the sensitivity of growth factors to enzymatic degradation and develops a specific cellular-molecular environment that regulates tissue regeneration [3]. The bone callus, which forms on the surface of the implant and is mainly composed of fibrin, collagen and elastin molecules, provides the basis for the formation of the tissue structure of the bone regenerate through the migration and adhesion of stem cells. The formation of a stable connection between the bone tissue of the recipient bed and the surface of the implant with its further osteointegration occurs [3]. Artificial surfaces activate the hemostasis system and hypercoagulation processes, creating the risk of thromboembolism of the main arteries. To prevent the development of postoperative complications caused by hypercoagulable syndrome, therapeutic strategies with the use of anticoagulant drugs, including anticoagulants (heparin, warfarin) are used [1]. It should be noted that the use of direct anticoagulants (especially heparin) limits the normal formation of a fibrin clot in vivo, which slows the migration of mesenchymal stromal cells (MSCs) into the structure of the developing callus and also contributes to the disruption of the processes of osseointegration of implants and osteoreparation. Several research groups report that glycosaminoglycans, especially heparin, are potent co-stimulants of osteogenic signaling pathways. For example, L. Ling et al. (2010) found that heparin activates the Wnt signaling pathway through physical interaction with the Wnt3a ligand, thereby enhancing the differentiation of MSC to osteoblasts [4].

The aim of this study was to investigate the effect of heparin at pharmacological concentrations on the expression of membrane markers of stemness of MSCs in human adipose tissue and their ability to differentiate in the osteogenic direction under in vitro culture conditions.

MATERIALS AND METHODS

MSCs were obtained from human lipoaspirate (approval no. 7 dated 12.09.2015 from the local ethics committee of IKBFU Innovation Park) and met the minimum criteria for MSC: cell viability greater than 95 %; adhesion to the surface of the culture plastic; expression of the membrane markers CD105, CD73 and CD90 in combination with minimal expression (≤2 %) of CD45/CD34 on the cell surface; the ability to differentiate in three directions (osteogenic, chondrogenic and adipogenic) [5]. To assess the morphofunctional state of cells cultured in the presence of heparin, 2 experimental groups were formed: 1) cultivation of MSC in the presence of heparin at a therapeutic concentration (1.3 IU/ml); 2) cultivation of MSC in the presence of heparin at a toxic concentration (13 IU/ml). A model of MSC cultivation in a complete culture medium based on DMEM/F12 served as a control. To assess the response of cells in the presence of heparin (Belmedpreparaty, Belarus), MSC (1×10^5 cells/ml) were cultured in sterile 12-well apartment-bottomed plastic plates (Orange Scientific, Belgium) in 2 ml of complete culture medium (PPS) (90 % DMEM/F12 (1:1) (Gibco Life Technologies, USA), 10 % FBS (Sigma Aldrich, USA), 50 mg/l gentamicin (Invitrogen, UK), 280 mg/l L-glutamine (Sigma Aldrich, USA) for 14 days at 37 °C, 100 % humidity, with 5 % CO2 with a change of medium every 3–4 days. 130-095-198 (Miltenyi Biotec, USA) according to the manufacturer's protocol, after which the cells were analyzed on a MACS Quant flow cytometer (Miltenyi Biotec, Germany). The data were processed using KALUZA Analysis software (Beckman Coulter, USA).

To evaluate the change in mRNA transcription of osteodifferentiation genes (BMP2, BMP6, ALPL, RUNX2, BGLAP and SMURF1), total RNA was isolated from cells using an aqueous solution of phenol and guanidine isothiocyanate (ExtractRNA kit, Evrogen, Russia) according to the manufacturer's protocol and the reverse transcription reaction (MMLV kit, "Evrogen", Russia) was performed. Multiplex PCR analysis with specific TaqMan probes (Beagle, Russia) was performed to determine the relative mRNA expression of the genes. PCR was performed using qPCRmixHS reagents (Evrogen, Russia) and primers at a concentration of 10 pM. 4 µL of cDNA was used as a template, and the RPLPO gene served as a reference gene. The relative expression of the mRNA genes was determined using the modified Pfaffl formula. Statistical processing of the obtained data was performed using statistical description methods and statistical hypothesis testing methods. The sample was tested for normality using the Kolmogorov-Smirnov test. Since the data did not follow the law of normal distribution, the median (M), 25 % quartile (Q1) and 75 % quartile (Q3) were determined for descriptive statistics. To evaluate the statistical significance of the differences, the nonparametric Wilcoxon T-test was used for dependent samples and the Mann – Whitney U-test for independent samples. Statistical analysis of the results was performed using the software package Graph Pad Prism version 8.0.1 (Graph Pad Software Inc, San Diego, CA, USA). Differences were considered statistically significant at a significance level of $p < 0.05$.

RESULTS AND DISCUSSION

MSC's stemness markers

According to the results of flow cytometry, in the cultures with the addition of heparin at a concentration of 13 IU/ml, the content of cells carrying the surface marker CD90 increased by 9 % compared to the control values

Table 1. Expression of surface markers on MSC after 14 days of cultivation with heparin. For each group, the mean characteristics of the sample were calculated: the median (M), the first and third quartiles (Q1 and Q3)

Study groups, n=3	Proportion of living cells, %	CD90⁺	CD73⁺	CD105⁺
Control group (MSC w/o heparin)	80,4 (78,75–84,46)	78,97 (77,56–81,39)	78,22 (74,03–78,97)	17,93 (13,2–23,72)
MSC + heparin 1,3IU/ml	82,08 (80,26–82,58)	79,79 (75,15-85,02)	84,59 (84,24–95,72)*	18,97 (16,36–22,26)
MSC + heparin 13IU/ml	77,57 (64,31–79,13)	87,91 (81,57–88,21)*	87,26 (84,6–88,21)*	34,43 (14,14–35,22)

Note. * Statistical differences ($p \leq 0.05$) compared to the control group according to the Mann – Whitney U test.

($p \leq 0.05$) (Table 1). Moreover, a statistically significant increase in the number of CD73⁺ cells was observed after the addition of heparin at concentrations of 1.3 IU/ml (by 6 %) and 13 IU/ml (by 9 %). In a study by H. Ali et al (2015), an increase in the percentage of cells expressing the markers CD90 and CD73 indicated a poorly differentiated state of the cell culture [6]. In an experiment by Moraes D.A. (2016), a decrease in CD90 expression was associated with osteogenic and adipogenic differentiation of MSCs [7, 8]. CD90 is thought to control the differentiation of MSCs by acting as an obstacle to adherence during differentiation, thus helping to maintain the "stemness" of the cell culture under study [7]. However, there are also data suggesting that the lack of CD90 on the cell surface is associated with a decrease in the expression of proosteogenic factors with a concomitant increase in the expression of inhibitors of differentiation pathways (e.g. the Wnt pathway) [9]. The data obtained in this experiment may indicate a possible role of CD90 as a temporal marker for the early differentiation of MSC in the osteoblastic direction. CD73 has been identified as an underlying mechanism for the conversion of adenosine with its further effect on reducing the excessive reactivity of platelets during the activation of blood coagulation [10]. Thus, cells with CD73 on their surface are able to independently exert an anticoagulant effect. According to the data obtained, heparin at the concentrations studied (1.3 IU/ml; 13 IU/ml) contributes to a statistically significant increase (compared to the control group; table 1) in the number of CD73⁺ cells in MSC culture. Thus, as a direct anticoagulant, heparin molecules are also able to exert an indirect anticoagulant effect at the cellular level through an increase in the proportion of CD73⁺ MSC cells.

Expression of genes for osteodifferentiation

It was found that the relative mRNA expression of BMP2 and RUNX2 genes remained at the level of control values when heparin was added to the MSC culture at a therapeutic concentration (1.3 IU/ml) (figure 1). At the same time, the anticoagulant at a concentration of 13 IU/ml statistically significantly (3-fold) increased the transcription of their mRNA (figure 1).

W. Jang (2011) showed that BMP2 activates Smad1/5/8 signaling, thereby regulating the transcription of osteogenic genes and initiating the expression of RUNX2 gene mRNA [11]. Therefore, the increase in relative mRNA expression of BMP2 and RUNX2 genes in the presence of heparin at a toxic concentration (13 IU/ml) may indicate the possible involvement of heparin in the early stages of initiation of genes responsible for osteogenic differentiation of MSCs in vitro.

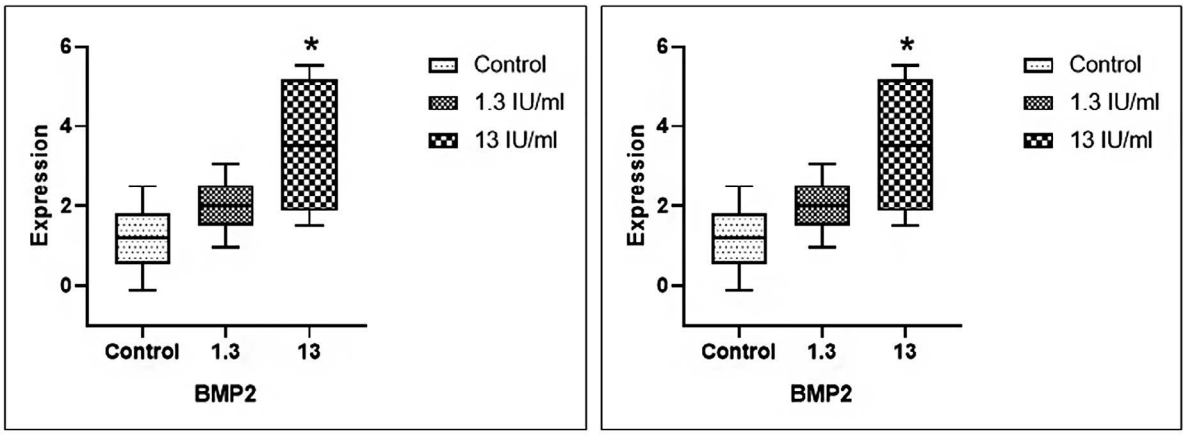

Figure 1. Level of relative expression of mRNA of RUNX2 and BMP2 genes associated with differentiation and maturation of MSC in osteogenic direction after 14 days of cultivation. * Significant differences compared to control cell culture (w/o heparin)

In the experiment, it was found that the addition of heparin at a high dose (13 IU/ml) had no effect on the level of relative expression of mRNA of the genes BMP6 and ALPL compared with the values obtained when the control group was evaluated (figure 2). No statistical differences were found in the level of relative expression of mRNA of BMP6 gene when heparin was added at a concentration of 1.3 IU/ml (figure 2).

It is known that BMP6, together with BMP2, are endogenous regulators of human osteoblast differentiation and play a key role in skeletal development [12]. Although statistical analysis did not reveal significant differences compared to control, there is a tendency for an increase in BMP6 mRNA transcription (figure 2). The authors of numerous studies reported a more significant role of BMP6 in the induction of osteogenic differentiation in vitro compared to the effect of BMP2 [13]. Moreover, J. Z. Li et al (2003) demonstrated that the induction of alkaline phosphatase by BMP6 adenoviruses was higher than that by BMP2 adenoviruses [14].

In turn, heparin at a therapeutic concentration (1.3 IU/ml) statistically significantly increased the level of relative expression of ALPL gene mRNA compared to the values obtained when the control group was evaluated. ALPL induction, ALPL mRNA level and alkaline phosphatase activity (ALP) are considered reliable predictors of MSC differentiation into osteoblasts and the ability to form bone tissue [15].

Figure 3 shows that the addition of heparin at a high dose (13 IU/ml) resulted in a statistically significant increase in the expression of the mRNA of the BGLAP gene. BGLAP or osteocalcin is a small conserved extracellular matrix protein lacking collagen that is expressed during late osteoblast differentiation and is abundant in bone tissue. The function of osteocalcin is related to bone mineralization and synthesis of mineralized extracellular matrix [16]. S. Kannan showed that an increase in the expression of the mRNA of the BGLAP gene is associated with the readiness of cells for osteogenic differentiation, which was further confirmed by the early appearance of preosteoblastic cells in this

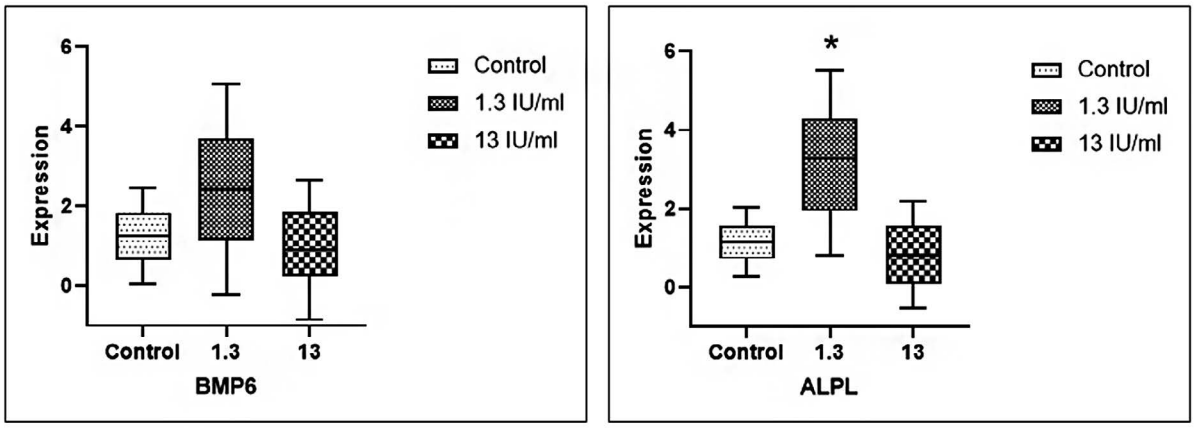

Figure 2. Level of relative expression of mRNA of BMP6 and ALPL genes associated with differentiation and maturation of MSC in osteogenic direction after 14 days of cultivation. * Significant differences compared to control cell culture (w/o heparin)

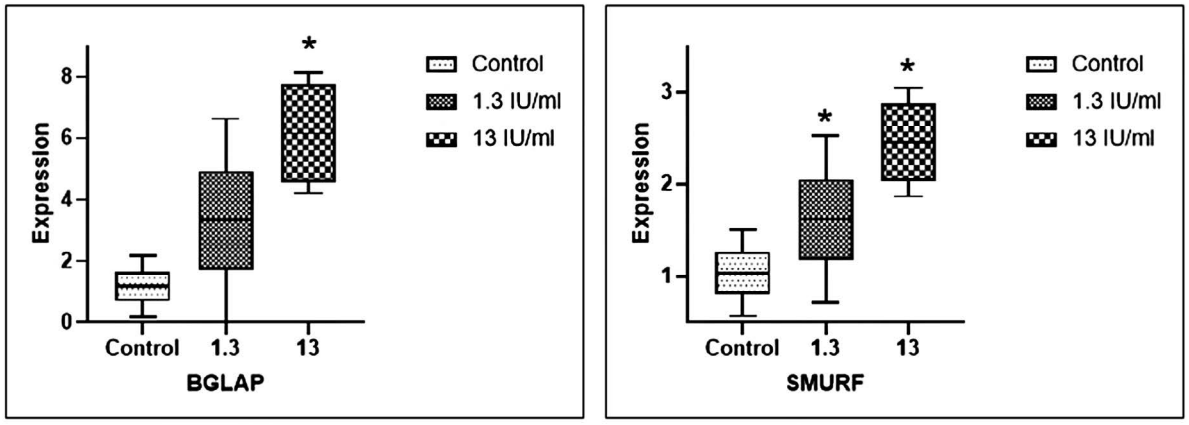

Figure 3. Level of relative expression of mRNA of BGLAP and SMURF1 genes associated with differentiation and maturation of MSC in osteogenic direction after 14 days of cultivation. * Significant differences compared to control cell culture (w/o heparin)

study [16]. The level of SMURF1 gene mRNA expression increased statistically significantly when heparin was added to the MSC culture at both therapeutic (1.3 IU/ml) and toxic (13 IU/ml) concentrations. It should be noted that the expression of SMURF1 gene mRNA was higher when heparin was added at a toxic concentration (figure 3). In 2003, L. Xing first described the function of SMURF1 in RUNX2 degradation and osteoblast function. He showed that overexpression of the mRNA of this gene induces proteasomal degradation of SMAD1 and RUNX2 proteins in osteoblast progenitor cells. Moreover, SMURF1 was shown to be the first E3 ligase identified in the BMP pathway as a negative regulator of bone cell function [17]. We mean here that the increased expression of SMURF1 gene mRNA is directly dependent on the increased expression of RUNX2 gene mRNA. Thus, negative regulation of MSC differentiation towards osteoblasts was induced in cells.

Thus, heparin at a therapeutic dose (1.3 IU/ml) significantly stimulates the expression of ALPL and SMURF1 of the MSC osteodifferentiation genes in vitro. In turn, the osteomodulatory effect of a high concentration of the anticoagulant (13 IU/ml) is evident with respect to the expression of BMP2, RUNX2, BGLAP and SMURF1 genes (figure 1–3). The differential epigenetic effect of different doses of anticoagulant molecules remains to be deciphered.

CONCLUSION

The addition of heparin to the MSC culture of human adipose tissue in vitro leads to an increase in the percentage of $CD90^+$ cells (at a concentration of 13 IU/ml) and $CD73^+$ cells (at concentrations of 1.3 IU/ml and 13 IU/ml) after 14 days. These results may indicate the stimulatory effect of heparin on the maintenance of the stem state of the MSC culture with the readiness of the cells to differentiate in the osteoblastic direction. It should be noted that heparin can exert an independent anticoagulant effect in MSC culture by increasing the number of $CD73^+$ cells.

In general, heparin showed a stimulatory effect on the expression of mRNA of various osteodifferentiation genes at both therapeutic (1.3 IU/ml) and high (13 IU/ml) concentrations. At the same time, a dose-dependent increase in mRNA expression of the SMURF1 gene was observed at both doses of heparin. This gene encodes an E3 ubiquitin-protein ligase that promotes the degradation of RUNX2, which may indicate a transitional state of the MSC culture between the stem state and readiness for osteogenic differentiation.

The data we obtained argue for the complex relationships between the coagulation and anticoagulation systems in the formation of hematomas and the regulation of the processes of osteodifferentiation of MSC. In this regard, the results are interesting for regenerative medicine technologies. On the other hand, they should be taken into account in postoperative treatment of patients with a high risk of postoperative thrombosis in arthroplasty of large joints and osteosynthesis of fractures.

REFERENCES

1. Labarrere C. A., Dabiri A. E., Kassab G. S. Thrombogenic and Inflammatory Reactions to Biomaterials in Medical Devices. *Frontiers in Bioengineering and biotechnology*. 2020;8:23. DOI: 10.3389/fbioe.2020.00123.
2. Gorbet M. B., Sefton M. V. Biomaterial-associated thrombosis: roles of coagulation factors, complement, platelets and leukocytes. *Biomaterials*. 2004;25(26):5681–5703. DOI: 10.1016/j.biomaterials.2004.01.023.
3. Chen Q. Potential role for heparan sulfate proteoglycans in regulation of transforming growth factor-beta (TGF-beta) by modulating assembly of latent TGF-beta-binding protein-1. *Journal of Biological Chemistry*. 2007;282(36):26418–26430. DOI: 10.1074/jbc.M703341200.
4. Ling L. Synergism between Wnt3a and Heparin Enhances Osteogenesis via a Phosphoinositide 3-Kinase/Akt/RUNX2 Pathway. *Journal of Biological Chemistry*. 2010;285(34):26233–26244. DOI: 10.1074/jbc.M110.122069.
5. Christodoulou I., Goulielmaki M., Devetzi M., Panagiotidis M., Koliakos G., Zoumpourlis V. Mesenchymal stem cells in preclinical cancer cytotherapy: a systematic review. *Stem Cell Research & Therapy*. 2018;9(336). DOI: 10.1186/s13287-018-1078-8.
6. Ali H., Al-Yatama M. K., Abu-Farha M., Behbehani K., Al Madhoun A. Multi-Lineage Differentiation of Human Umbilical Cord Wharton's Jelly Mesenchymal Stromal Cells Mediates Changes in the Expression Profile of Stemness Markers. *PLoS One*. 2015;10(4). DOI: 10.1371/journal.pone.0122465.
7. Moraes. D. A. A reduction in CD90 (THY-1) expression results in increased differentiation of mesenchymal stromal cells. *Stem Cell Research & Therapy*. 2016; 7. DOI: 10.1186/s13287-016-0359-3.
8. Sun Q., Nakata H., Yamamoto M., Kasugai S., Kuroda S. Comparison of gingiva-derived and bone marrow mesenchymal stem cells for osteogenesis. *Journal of Cellular and Molecular Medicine*. 2019;23(11):7592–7601. DOI: 10.1111/jcmm.14632.
9. Picke A. K. Thy-1 (CD90) promotes bone formation and protects against obesity. *Science Translational Medicine*. 2018;10(453). DOI: 10.1126/scitranslmed.aao6806.
10. Netsch P. Human mesenchymal stromal cells inhibit platelet activation and aggregation involving CD73-converted adenosine. *Stem Cell Research & Therapy*. 2018;9(1):184. DOI: 10.1186/s13287-018-0936-8.
11. Jang W. G. BMP2 protein regulates osteocalcin expression via Runx2-mediated Atf6 gene transcription. *Journal of Biological Chemistry*. 2012;287(2):905–915. DOI: 10.1074/jbc.M111.253187.
12. Ye F. The role of BMP6 in the proliferation and differentiation of chicken cartilage cells. *PLoS One*. 2019;14(7). DOI: 10.1371/journal.pone.0204384.
13. Cai C., Wang J., Huo N., Wen L., Xue P., Huang Y. Msx2 plays an important role in BMP6-induced osteogenic differentiation of two mesenchymal cell lines: C3H10T1/2 and C2C12. *Regenerative Therapy*. 2020;14:45–251. DOI: 10.1016/j.reth.2020.03.015.
14. Li J. Z. Osteogenic potential of five different recombinant human bone morphogenetic protein adenoviral vectors in the rat. *Gene therapy*. 2003;10(20): 1735–1743. DOI: 10.1038/sj.gt.3302075.
15. Ofiteru A. M. Qualifying Osteogenic Potency Assay Metrics for Human Multipotent Stromal Cells: TGF-β2 a Telling Eligible Biomarker. *Cells*. 2020; 9(12): 2559. DOI: 10.3390/cells9122559.
16. Kannan S., Ghosh J., Dhara S. K. Osteogenic differentiation potential of porcine bone marrow mesenchymal stem cell subpopulations selected in different basal media. *Biology Open*. 2020;9(10). DOI: 10.1242/bio.053280.
17. Zhu B., Xue F., Zhang C., Li G. LMCD1 promotes osteogenic differentiation of human bone marrow stem cells by regulating BMP signaling. *Cell Death & Disease*. 2019;10(9):647. DOI: 10.1038/s41419-019-1876-7.